Int. J. Radiat. Biol., Vol. 84, No. 4, April 2008, pp. 325–335

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Altered blood chemistry and hippocampal histomorphology in adult rats following prenatal exposure to physiologically-patterned, weak (50–500 nanoTesla range) magnetic fields

L. S. ST-PIERRE[1], A. MAZZUCHIN[2,3], & M. A. PERSINGER[1]

[1]*Behavioral Neuroscience Laboratory and* [2]*Department of Biology, Biomolecular Science Program, Laurentian University, Sudbury, and* [3]*School of Health Sciences and Emergency Services, Cambrian College of Applied Arts and Technology, Greater Sudbury, Ontario, Canada*

(Received 4 March 2007; revised 23 November 2007; accepted 2 January 2008)

Abstract

Purpose: To discern changes in blood chemistry, cerebral sizes, and hippocampal cytomorphology in adult male and female albino Wistar rats that had been exposed during their entire prenatal development to one of two patterns of magnetic fields and one of four intensities: Very low 5–20 nT; low 30–50 nT; medium 90–580 nT; and high 590 nT to 1.2 microT.

Materials and methods: A total of 48 pregnant females were exposed to either a repetitive frequency-modulated magnetic field or to a complex sequence of 50, 200-msec physiologically-patterned fields. As adults blood, cerebral, and histomorphological data were obtained from the 137 rats that had been exposed to one of these eight conditions.

Results: Compared to other groups, adult rats that had been exposed prenatally to the physiologically-patterned magnetic fields at the low (30–50 nT) and medium (90–580 nT) intensities exhibited peak elevations of aminotransaminase, glucose, and uric acid. Numbers of cytometric anomalies were also significantly elevated within regions of the hippocampus known for neuronal neogenesis in adults.

Conclusions: The results suggest that a common factor in cellular adhesion or plasticity might be permanently altered by prenatal exposure to a narrow intensity of a series of physiologically-patterned magnetic fields.

Keywords: *Magnetic fields, blood chemistry, hippocampus*

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Introduction

Pulsed magnetic fields, which have been shown to induce cellular transcription in dipteran salivary glands after 15 min of exposure (Goodman et al. 1983) and to modulate gene expression in myocardial cells (Ventura et al. 2000), may be more biologically effective than sine-waves (Huuskonen et al. 1993, Brent 1999, Zusman et al. 1990) when presented at comparable intensities (McKay et al. 2000). The term pulsed magnetic fields has been applied to configurations that occur intermittently rather than continuously and whose rise, plateau, and descent times may not be as symmetrical as sine-waves. Classic biological examples of pulsed magnetic fields would include 'burst-firing' cells within the hippocampus. They can exhibit complex-patterned pulses whose interpulse intervals change over time.

The importance of interstimulus pulse times is most clearly shown with long-term potentiation (LTP) in hippocampal slices where optimal effects are found when a primer pulse is followed approximately 170 msec later by a minimum of four rapid pulses (Rose et al. 1988). Continuous sine-wave applications do not produce this effect. When generated within the home environment, pulsed magnetic fields could be contributing factors for the induction of oncogenes Cfos ab1 and integrin alpha 5 beta 1 (Omura & Bro 1993) and have been correlated with the incidence of childhood leukaemia (Wertheimer & Leeper 1979). It may be relevant that power frequency (50 Hz, 60 Hz) fields at intensities encountered in some homes can increase ornithine decarboxylase activity to levels induced by the carcinogens in the mammary gland (Mevissen et al. 1995).

We have found that whole-body exposures to physiologically-patterned magnetic fields composed

Correspondence: Dr M. A. Persinger, Behavioral Neuroscience Laboratory, Department of Biology, Laurentian University, Sudbury, Ontario Canada P3E 2C6. E-mail: mpersinger@laurentian.ca

ISSN 0955-3002 print/ISSN 1362-3095 online © 2008 Informa UK Ltd.
DOI: 10.1080/09553000801953300

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of pulses whose temporal structures simulate patterns generated by cells or aggregates of cells within the brain itself can evoke powerful changes in behavior at intensities more than a hundred times less than those required for repetitive sine waves. Several studies (McKay et al. 2000, Stewart & Persinger 2000, Persinger 2003) have shown that very specific patterns of magnetic fields affect specific behaviors strongly correlated with neuroelectrical activity within different regions of the hippocampus whereas intensity-matched sine waves that reflect the 'averaged' frequency of the patterns did not produce any significant behavioral changes except at much higher (mT) intensities. The effective intensities for the complex fields were between 20 nT and 2000 nT, or 0.2 mG to 20 mG, well within the range of routine human exposure.

In the present study, we exposed rats during their entire prenatal development to one of two types of complex, weak magnetic fields whose structures were patterned after natural (Cherry 2002) physiologically-relevant activity. The first was a repetitive frequency-modulated pattern that has been shown to induce aggression (St-Pierre et al. 1998) and seizures in epileptic rats (Persinger & Bellanger-Chellew 1999) and analgesia in snails (Thomas et al. 1997). The second pattern was custom-constructed, from both theory (Wei 1969, Persinger & Koren 2007) and a general appreciation of physiological patterns, to affect development. It was called the complex-sequenced field because it contained a series of different patterns (see Figure 1) that have been shown to be behaviorally effective in adults. Rats that have been exposed during their entire prenatal development to this complex sequence magnetic field have shown qualitatively conspicuous increases in open field activity (Persinger et al. 2001) and much less proficient maze learning (McKay et al. 2002).

A component of the complex sequence field was a pattern of one pulse (primer) followed 173 msec later by four more pulses. This pattern was extracted exactly from the work of Rose et al. (1988) who found that electrical stimulation of hippocampal slices with this sequence produced reliable LTP. Whole body application of this LTP-promoting pattern as a magnetic field for 30 min after a conditioned fear task (whose integrity is associated with hippocampal function) has been shown to powerfully attenuate the response (McKay et al. 2000). We predicted that the temporal parameters intimately involved with a process essential to organization of the structure of memory formation might also affect the organization of the cells that ultimately contribute to this property within the developing hippocampus.

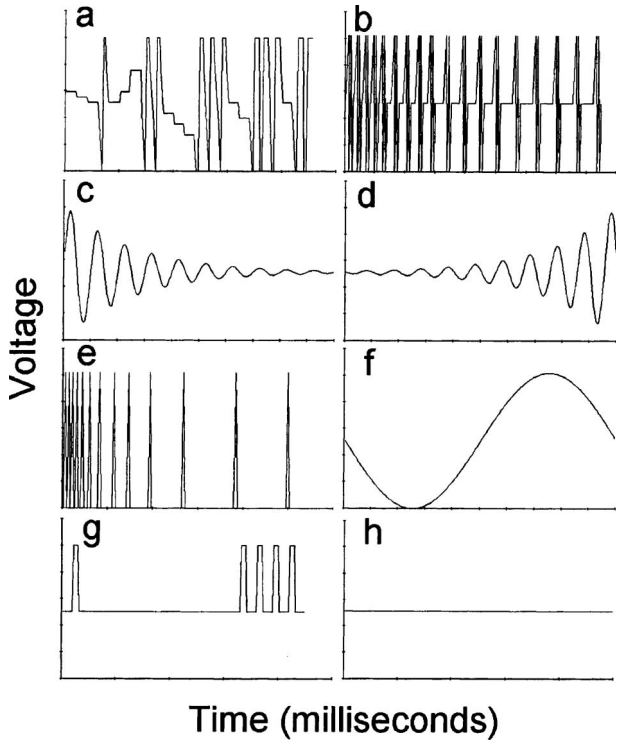

Figure 1. The different patterns (see Table I for sequence) that composed the complex sequence magnetic field. (a) Burst-firing, (b) frequency modulated (FM), (c) negative ring (n-ring), (d) positive ring (p-ring), (e) Fibonacci expansion, (f) 5 Hz sine wave, (g) LTP or long-term potentiation (positive polarity only), and (h) zero polarity. Voltage refers to values between −5 and +5 V from the computer-generated input to the coils through a digital-to-analogue converter. Time refers to the display of the patterns. The point durations were 1 msec.

This hypothesis was derived from our general interest in isolating the fundamental algorithms existing as gnomons (Ball 1999, Gazale 1999) within the organism that might contribute to both the positive and negative aspects of cellular plasticity and growth. We employ the term gnomon in a classical sense: It is any form (spatial or temporal) that, when added to some other form, results in a new form similar to the original. The purpose of the present study was to discern if exposures to a range of different intensities of the two shapes of magnetic fields during prenatal development would produce long-term changes in blood chemistry and specific (hippocampal) brain morphology.

Rather than employ homogeneous intensities of magnetic fields, we exposed the subjects to four separate ranges of intensities between 5 nT and 1.2 microT generated between two separated coils. Although the applications of more homogeneous magnetic fields from Helmholtz or Merritt coils have specific advantages to explore traditional mechanisms, the natural environment is more heterogeneous. The specific ranges of intensities were selected on the bases of their effectiveness in

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producing behavioral changes in adult rats and their prominence within human habitats.

Materials and methods

Animals

Wistar albino rats were obtained from Charles River Suppliers (Quebec). All protocols involved with maintaining, exposing, and termination of the rats had been approved by the local Animal Care Committee following the guidelines of the Canadian Council on Animal Care.

Blood chemistry. A total 32 rats (16 males, 16 females), 90 days of age, that had been exposed to one of the four intensities (four male; four females per intensity) were selected as subjects. Two males and two females were randomly selected from each of the two litters that had been exposed to each of the intensities.

Brain measurements and histology. An additional 105 male and female rats, approximately eight months old, were selected for measurements of cerebral size from all litters exposed (numbers in each condition in parentheses) to the frequency modulated field: Control (15), low intensity (15), medium intensity (13) and high intensity (13) and from all litters exposed to the complex sequenced field: Control (13) low (14), medium (11) and high (11).

Exposure system and EMF

In six separate blocks of experiments 48 breeder females, 90 to 100-day-old Wistar albino rats and 24 breeder males, 90 to 120-day-old male Wistar rats were housed in one of four equally sized compartments within the same transparent plastic cage whose dimensions were $140 \times 33 \times 33$ cm. There were two females and one male per compartment. The light intensity in each compartment ranged between 200 and 220 lux. The sound levels from the computer generating the fields were within 1 db of 54 db, as measured by a (Circuit City, InterTAN Canada Ltd., Head Office, 279 Bayview Drive, Barrie, ON, Canada) Sound Level Meter (Setting C), within each compartment.

At each end of the cage was a magnetic coil. The two coils were identical and were created by wrapping plastic cartons (milk crates) with 305 m of 30 AWG (Belden #9978) wire in a single layer (18 cm wide). The crates had been obtained from FREM (Charter Gate Clayfield Close, Moulton Park Northampton, NN3 6QF, UK) Corporation (no. 701-4) and were $38 \times 33 \times 27$ cm. The circuitry was designed so that only one coil would be activated for any given block of experiments.

Three of the blocks involved the employment of the complex sequence pattern (Figure 1). The sequence of its composition is shown in Table I. This sequence was presented for 10 sec every 50 sec, i.e., 10 sec on, 40 sec off. The other three blocks employed the continuous presentation of the frequency-modulated pattern (Figure 1b). For two of the blocks for each pattern the activated coil was at one end of the cage while for the other block the other coil was activated. This was done to insure that any effects were due to the applied magnetic fields rather than to peculiarities of one coil or the space in which the rats had been maintained. Because there was no statistically significant difference in the compartmental effects as a function of distance from either of the activated coils, the data were analyzed as a function of the distance from the activated coil.

The specific patterns of the magnetic fields were generated by converting columns of numbers, each containing a number between 0 and 255 into voltages by an XT personal computer. A value of 127 was 0 V while a value of 255 was +5 V and a value of 0 was −5 V. There were 9999 numbers for the complex sequence pattern and 849 numbers for the frequency-modulated pattern. These numbers were then converted by a custom-constructed device into voltages that were directed into one of the two coils. Because a changing electric field is associated with a changing magnetic field, a magnetic field was generated through the coil or solenoid. Other details have been published previously (St-Pierre et al. 1998).

Table I. The specific sequence (read from left to right and top, down) of patterns (see Figure 1) composing the complex-sequence field. The duration of each of the 50 presentations was 200 msec for a total of 10 sec once every 50 sec.

1	Burst	LTP	FM	LTP	Burst	LTP	FM	LTP
2	Burst	LTP	FM	LTP	Burst	LTP	FM	LTP
3	Neutral							
4	P-ring	LTP	N-ring	LTP	P-ring	LTP	N-ring	LTP
5	P-ring	LTP	N-ring	LTP	P-ring	LTP	N-ring	LTP
6	Neutral							
7	Fibonacci	LTP	5 Hz	LTP	Fibonacci	LTP	5 Hz	LTP
8	Fibonacci	LTP	5 Hz	LTP	Fibonacci	LTP	5 Hz	LTP

LTP, Long-term Potentiation; FM, Frequency modulation.

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The point durations (the time the voltage specified by a given number was maintained) were 3 msec for the frequency-modulated pattern and 1 msec for the complex sequence pattern. The rise time between successive points was 100 microsec. The 1 msec point duration for the complex sequence field was based on the theoretical work of Fong (1968), Wei (1969) and Persinger and Robb (1976). Fong considered each base of RNA to be an electric dipole. A pulse of 100 mV for 1 msec, which is long enough to act on the moving tape (RNA strand) for a distance of 0.5 nm (the width a base), would impart about 10^{-20} joules to a dipole. The theoretical and practical significance of the 1 msec and 3 msec point durations was based upon the derivations of Persinger and Koren (2007).

The value of 10^{-20} J is comparable to the stacking energy of one base and might be capable of 'unstacking' one base. According to Faraday's law the change in a magnetic field of 10 microT every 0.1 msec over a 0.01 m (1 cm) length would induce a voltage within this range. We reasoned that the different patterns contained with the complex sequence magnetic fields might statistically overlap with either the timing of the base pair additions for either DNA or RNA and as a result produce permanent changes in cellular systems vulnerable to the specific pattern. Subtle differences might be amplified during ontogeny. The mark:space ratio of 1:4 was employed to minimize the likelihood the progressive stages of cellular and biochemical ontogeny would habituate quickly to the applied field. The continuous presentation of the redundant frequency-modulated field was a control for this assumption.

All breeder females were exposed continuously to the fields from the time they were housed with their male mates to about 24 h after they gave birth (Persinger et al. 2001, St-Pierre & Persinger 2007). The males were removed eight days after their first exposure to the females. The ranges of intensities of the complex magnetic fields within the four chambers decreased as a function of distance from the activated coil. The four successive ranges, from either activated coil, were 590 – 1200 nT (12 mG), 90 – 580 nT, 30 – 50 nT and 5 – 20 nT. These values did not differ between the two patterns. In this paper these ranges defined the high intensity, medium intensity, low intensity, and very low intensity group, respectively. The rats from the 15 litters delivered in the frequency-modulated fields and 18 litters delivered in the complex sequence field were culled to four males and four females within 24 h (St-Pierre & Persinger 2007).

Analyses of variance for litter sizes indicated no statistically significant difference between either the patterns [$F(1,25) = 1.65$] or intensities [$F(3,25) = 1.78$] for total numbers of pups (grand mean = 11.9; SD [Standard Deviation] = 5.5). However there was a main effect between intensities for differences in numbers of male pups. There were significantly fewer male pups in litters exposed to the high intensity fields (M or Mean = 4.6, SD = 2.4) regardless of pattern compared to any of the less intense fields (M = 7.3, SD = 2.1; M = 6.9, SD = 2.0; M = 6.4, SD = 1.7, respectively). The means and standard deviations (in parentheses) for the numbers of female pups for each of the four intensities (high to low) were 7.6 (2.9), 6.8 (2.2), 7.1 (2.4) and 7.4 (1.4).

Blood chemistry

The mothers of the subjects had been exposed to the complex sequence field. We selected these rats because of their conspicuous anomalous behaviors in other tasks (McKay et al. 2003) that were not observed in the rats exposed to the simple frequency-modulated field. After weaning the rats had been maintained in plastic cages, 4 rats of the same sex per cage. However unlike previous experiments the rats were housed within the same room in which they were exposed to the fields. The sounds from the computer that had generated the fields were continuous through their development. There were no experimental fields generated.

When the rats were 3 months old each rat was exposed to carbon dioxide for about one min. While anesthetized, each rat was taken to the operating room. The rat was decapitated and trunk blood was collected. Plastic beads were added to help remove serum. After 20 min, the blood was centrifuged for 10 min. The serum was extracted by micropipette, placed in small containers, and refrigerated at 4°C.

Within 6 – 20 h after blood collection, the serum was analyzed automatically at a local hospital's blood chemistry laboratory for the following constituents (see Griffith 1988): AST (the old SGOT or serum glutamic oxalic transaminase, now aspartate aminotransferase), glucose, BUN (blood urea nitrogen), creatinine, sodium, potassium, chloride, calcium, phosphorus, LDH (lactate dehydrogenase), ALP (alkaline phosphatase), ALT (once called SGPT, now alanine aminotransferase), albumin, triglycerides, cholesterol, and T4 (thyroxine). A total of 32 rats, four males and four females randomly selected from each of the four intensities, were measured.

Brain measurements and histology

The brains of 105 male and female rats not involved in the blood chemistry study were removed following death by exposure to carbon dioxide. The length and the width of the cerebrums were measured by vernier

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calibers to the nearest 0.1 mm. The brains for 38 rats (19 from the frequency-modulated field, 19 from the complex sequence field) were randomly selected from this pool and fixed in ethanol-formalin-acetic acid (Sigma, Oakville, ON, Canada; Commercial Alcohols, Toronto, ON, Canada). Because we expected subtle effects, six brains from rats (three male, three female) that had been exposed to the sham fields during the same period and were age-matched were also processed as 'colony controls'. Near-equal numbers of males and female brains from each pattern for each of the four intensities were processed for histological analysis. Ten micrometer coronal sections were kept from every 20 sections from the cerebrum between the posterior and anterior commissures. Ten micrometer sagittal sections were obtained from the medial to midlateral portion of the cerebrum. Stains (all obtained from Fisher Scientific, Ottawa, ON, Canada) included toluidine blue O, haematoxylin and eosin (Persinger 1979), periodic-acid-Schiff (PAS) and a myelin stain (Humanson 1967). Sections were examined at 10× for perspective through a trisimplex projector at 100×, 400× and 1000× (oil) magnifications by light microscopy.

The following qualitative indicators were ranked on a semi-quantitative scale (0, not present, to 2, obvious to anyone): (i) The length of the dentate gyrus more than half the width of Ammon's Horn; (ii) the scatter of cells within (hilar) CA4 (CA = cornu ammonis or Ammon's horn) more than twice the width of pyramidal cell lines from CA3; (iii) the relative density of pyramidal cells scattered between the CA3-CA4 line and the surrounding granule cell layer of the dentate; (iv) the numbers of anomalies within CA3 and CA2; (v) the presence of pyramidal cells within the granule cell layer of the dentate gyrus; and (vi) the numbers of frank anomalies, such as ectopic tissues, within the region. In addition to the above indicators, we employed the criteria and cellular descriptions of Mathern et al. (1995), Scharman et al. (2000), and Fournier et al. (2007). The values were summed for a single anomaly score for each rat. In addition the presence of an oddly shaped hilar region or the granular layer that defined it was also indicated. The histomorphological scores were assigned by the third author who was not familiar with the treatment histories of the brains but had 35 years of experience with brain structures and light microscopy.

Statistical analyses

For brain measurements three-way analyses of variance were completed as a function of pattern, intensity, and sex. The presence of subtle morphological anomalies was assessed by two way (pattern, intensities) analyses of variance. The specific post hoc tests (Tukey, $p < 0.05$) were determined by the results. One way analyses of variance and polynomial analyses, to detect the functional relationships between prenatal intensity and the blood measures, were completed for each variable. All analyses involved SPSS software on a VAX 4000 computer.

Results

Analyses of variance for body weight for all rats for which this measure was taken ($n = 89$ of the 105) at death as a function of sex, pattern, and intensity showed that males were significantly heavier than females [$F(1,73) = 460.997$, $p < 0.001$; $\eta = 0.73$]. The significant interaction between magnetic field pattern and sex [$F(1,73) = 34.24$, $p < 0.001$] was due the males that had been prenatally exposed to the complex sequence fields being significantly heavier ($M = 758$ gm, $SD = 76$ gm) than those exposed to the frequency-modulated field ($M = 498$ gm, $SD = 67$ gm) while this was not evident for the females ($M = 428$, $SD = 68$ gm; $M = 384$, $SD = 32$ gm). The weak but statistically significant interaction between pattern and intensity [$F(3,73) = 2.65$, $p < 0.05$] was due in large part to significantly heavier weights for both sexes that had been exposed prenatally to the three highest intensities of the complex sequence fields compared to the 5–20 nT fields whereas there was no significant change over intensities for those rats that had been exposed to the simple, frequency-modulated field.

Blood chemistry

Except for AST levels, analyses of variance for each of the blood measures as a function of sex and intensity range for rats that had been exposed prenatally to the complex sequence magnetic fields demonstrated no statistically interaction between sex and intensity. Consequently the values for males and females for the blood measures shown in Table II were combined. The major source of the interaction [$F(3,24 = 3.40$, $p < 0.05$] between sex and intensity for AST levels according to post hoc analysis was due to the elevated values for males exposed to the 90–580 nT field ($M = 291$) compared to the males or females exposed to the other three intensities or the females exposed to the 90–580 nT intensity (all Ms between 153 and 195).

One way analyses of variance (all $dfs = 3,27$) showed statistically significant differences between intensities for AST ($F = 4.60$, $p < 0.01$), glucose ($F = 3.33$, $p < 0.05$), creatinine ($F = 7.35$, $p < 0.001$), calcium ($F = 3.36$, $p < 0.05$), ALT ($F = 5.44$, $p < 0.01$), albumin ($F = 3.59$, $p < 0.05$), uric acid ($F = 4.53$, $p < 0.05$), and triglycerides

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Table II. Means and standard deviations for the blood measures (using standard abbreviations) from rats exposed during the prenatal development to the four intensities of the complex sequenced magnetic field. There were 8 rats (4 males, 4 females) per intensity.

	Intensity							
	Control		Low		Medium		High	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AST (U/l)	160[a]	37	176[a]	43	243[b]	70	175[a]	36
Glucose (mg/dl)	12[a]	3	11	2	10[b]	1	13[a]	2
BUN (mg/dl)	8.5	1.1	8.3	0.7	8.0	1.2	8.1	0.9
Creatinine (md/dl)	22[a]	6	24	2	24	4	31[b]	4
Sodium (mEq/l)	150	1	150	1	150	2	149	3
Potassium (mEq/dl)	7.9	1.2	7.6	0.7	7.5	0.9	7.5	0.7
Chloride (mEq/dl)	101	0.7	101	1.1	101	2.1	98	2.1
Calcium (mg/dl)	3.3[a]	0.2	3.0[b]	0.1	3.2	0.1	3.2	0.1
Phosphorus (mg/dl)	3.7	0.2	3.5	0.2	3.3	0.4	3.4	0.3
LDH (U/l)	675	152	908	382	966	328	801	246
ALP (U/l)	167	39	206	37	176	47	186	43
ALT (U/l)	65[a]	9	78	13	92[b]	19	87[b]	12
Albumin (g/dl)	45	4	42[a]	1	46[b]	3	44	2
Uric Acid (mg/dl)	170[a]	77	111	16	87[b]	24	146[a]	52
Triglyceride (mg/dl)	3.6	1.2	3.4	1.1	1.9	0.5	2.1	0.8
Cholesterol (mg/dl)	1.6	0.2	2.1	0.4	1.9	0.5	2.1	0.8
Thyroxine (pmol/l)	40.7[a]	7.1	41.5	8.6	42.9	7.3	48.3[b]	6.8

a vs. b, $p < 0.05$.

Table III. Means (M) and standard deviations (SD) for the brain weights, cerebral lengths, and cerebral widths of adult rats that had been prenatally exposed to various intensities of either the frequency modulated (FM) or complex-sequenced (CS) field.

	Intensity							
	Control		Low		Medium		High	
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD
				Male brain weights (g)				
FM Pattern	2.07[a]	0.05	2.13[b]	0.06	2.05[a]	0.11	2.05[a]	0.05
CS Pattern	2.17[b]	0.03	2.25[b]	0.12	2.17[b]	0.09	2.17[b]	0.11
				Female brain weights (g)				
FM Pattern	1.89[a]	0.08	1.91[a]	0.07	1.95	0.11	1.90[a]	0.07
CS Pattern	1.95	0.05	1.98	0.10	2.05[b]	0.06	1.97	0.15
				Male cerebral lengths (mm)				
FM Pattern	15.2	0.4	15.8	0.6	14.1[a]	0.4	15.2[a]	0.8
CS Pattern	15.3[a]	0.2	16.4[b]	0.3	16.2[b]	0.4	15.8	0.6
				Female cerebral lengths (mm)				
FM Pattern	14.3	0.8	14.6	0.7	14.8	0.8	14.5	0.9
CS Pattern	14.1	0.8	14.7	0.4	14.9	0.8	14.4	0.5
				Male cerebral widths (mm)				
FM Pattern	15.1[a]	0.4	15.6[b]	0.3	15.0[a]	0.2	15.4	0.4
CS Pattern	15.2[a]	0.4	16.1[b]	0.5	15.8[b]	0.8	15.2[a]	0.7
				Female cerebral widths (mm)				
FM Pattern	14.7[a]	0.5	14.8[a]	0.4	14.7[a]	0.7	14.9[a]	0.3
CS Pattern	15.1[a]	0.5	15.2[a]	0.4	16.0[b]	0.6	15.2[a]	0.8

a vs. b, $p < 0.05$.

($F = 6.71$, $p < 0.001$). Post hoc tests indicated that the significant differences for AST, glucose, and uric acid were due to the rats that had been exposed to the medium (90 – 580 nT) intensity. Compared to the other groups, these rats showed higher levels of AST and lower levels of glucose and uric acid.

Trend (polynomial) analyses showed statistically significant linear relationships between intensity of the prenatal fields and the values of the measures were noted for creatinine, alanine aminotransferase (ALT), triglycerides, and thyroxine (Fs about 5.00, $p < 0.05$). Whereas the creatinine and ALT values

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increased with prenatal intensity triglycerides decreased with prenatal intensity. Cholesterol levels revealed a quadratic relationship ($F = 4.26$) because of the elevated values for rats exposed to the 30–50 nT intensities compared to the other ranges. The only significant gender differences were higher levels of BUN [$F = 26.98$, $\eta = 0.70$], phosphorous [$F = 11.98$, $\eta = 0.49$], albumin [$F = 5.16$, $\eta = 0.38$] and T4 [$F = 16.79$, $\eta = 0.59$] in males compared to females.

Brain weights

The males that had been exposed prenatally to the complex sequence fields showed significantly (all dfs = 1,41) greater cerebral lengths ($F = 7.78$) and widths ($F = 5.26$) and heavier brain weights ($F = 37.55$) compared to the rats that had been exposed to the frequency-modulated fields. A main effect of intensity of the field, regardless of pattern, was found for the lengths of the cerebrums [$F(3,33) = 3.91$, $p = 0.01$], the widths of the cerebrums [$F = 3.62$, $p < 0.05$] and the total weights of the brains ($F = 3.24$, $p < 0.05$).

Post hoc analysis indicated that the brains of the males that had been exposed to the low intensities (for either pattern) were heavier, longer, and wider than the brains from the other conditions. The statistically significant differences between intensities for the cerebral lengths ($F = 3.31$), widths ($F = 3.31$) and brain weights ($F = 3.31$) were still apparent after covariance for body weight. Analysis of the residuals between the observed and the expected values also showed that male rats exposed to the low intensities (30–50 nT) fields, regardless of patterns, exhibited heavier and larger brains than expected by their body weights.

For the females (all dfs [degrees of freedom] = 1,48), two-way analysis of variance showed that the brain widths ($F = 14.05$) were greater and the brains ($F = 21.73$) were significantly heavier for the rats that had been exposed to the complex sequence fields compared to the frequency-modulated fields. Covariance for body weight did not change these effects appreciably.

Hippocampal histology

The major conspicuous and most systematic qualitative effect occurred within the hippocampal formation at the level of the ventromedial hypothalamus. All of the cellular anomalies occurred within the CA3/CA4 (hilar) region. Two-way analysis of variance of the total histological anomaly score as a function of pattern and intensity showed that the rats that had been exposed to the complex sequence field displayed more total anomalies [$F(1,30) = 3.59$, $p < 0.05$; $\eta = 0.39$] than those exposed to the repetitive frequency-modulated field. According to the post hoc analysis the statistically significant differences in anomaly scores between the intensities of exposure [$F(3,30) = 3.59$, $p < 0.05$; $\eta = 0.44$] were due to the greater numbers of anomalies in the rats exposed to the low intensity (30–50 nT) field compared to rats that had been exposed to the other intensities.

However there was a strong statistically significant interaction between the type of field pattern and the intensity of the fields [$F(3,30) = 4.82$, $p < 0.005$]. The post hoc analysis, as shown in Figure 2, indicated that the primary source of the interaction was due to the marked increased incidence of anomalies within the rats exposed to the low intensity, complex sequence magnetic fields relative to those exposed to the reference (5–20 nT) region, the high intensity fields or the colony controls ($n = 6$) that had been exposed to sham fields.

Separate analysis of the rats exposed to the frequency-modulated fields only revealed no statistically significant differences in incidence of anomalies as a function of prenatal intensity [$F(3,15) = 1.41$, $p > 0.05$). Only the rats that had been exposed to the complex sequence field showed the significant intensity differences [$F(3,15) = 5.04$, $p < 0.01$; $\eta = 0.70$] that was due to the larger numbers of anomalies within the rats exposed to the low intensity. Polynomial analysis demonstrated that the effect deviated significantly from a linear relationship [$F(2,15) = 6.58$, $p < 0.001$] and exhibited a statistically significant quadratic function [$F(1,15) = 9.70$, $p < 0.001$]. Male brains that had

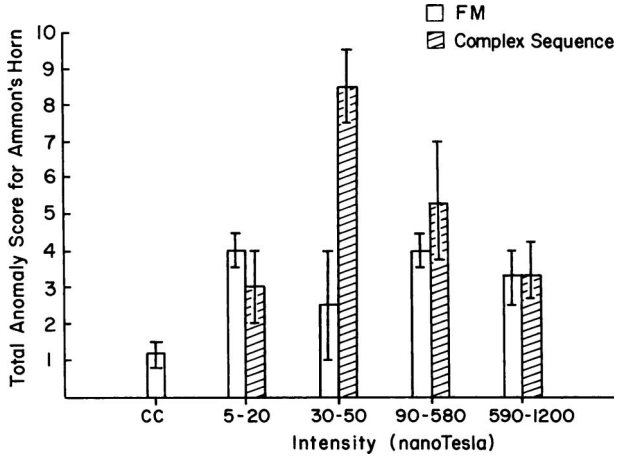

Figure 2. Total anomaly scores within the region of the dentate gyrus and the CA3/CA4 interface for rats that had been exposed during their prenatal development to various intensities of either the repeated frequency-modulated field or complex-sequenced patterns. Colony controls (age-matched rats from other studies) are shown for comparison. Vertical bars equal +/− 1 standard error of the mean.

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been exposed to this intensity and pattern exhibited more anomalies (by a factor of 2–3) than the female brains.

The most typical histomorphological changes involved irregular arrangements of cells or their distributions. The effect was most obvious in rats that had been exposed to the low intensity (30–50 nT; 0.3–0.5 mG) fields of the complex sequence patterns. All of the six rats sampled that had been exposed to this condition from six different litters and all three blocks exhibited this anomaly.

The extracelluar background or ground substance and the myelinated regions at this level around the hippocampus appeared qualitatively different for the rats that had been exposed to the complex sequence fields. Sections of the brains from the different intensities and patterns, stained with Luxol Fast Blue, PAS and Toluidine Blue O showed more vacuoles within the white matter of the fimbria. These vacuoles were only apparent in rats that had been exposed to complex sequence field, particularly the low and medium intensities, compared to the frequency-modulated fields. Their fimbrias were also more irregular and the typical rows of nuclei of oligodendroglia were less organized.

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Discussion

Prenatal exposure to either a redundant frequency-modulated magnetic field or the complex sequence field significantly increased the numbers of anomalies within the CA3/CA4 region of the hippocampal formation relative to colony controls. The numbers of anomalies were about twice as frequent in the hippocampi of rats that had been exposed to the low intensity or the 30–50 nT (0.3–0.5 mG) complex sequence magnetic fields compared to other intensities or any of the intensities from the frequency-modulated field. These anomalies occurred in the same region associated adult neuronal neurogenesis due to seizures in rats (Scharman et al. 2000) and temporal lobe epileptic patients (Mathern et al. 1995). In another experiment St-Pierre and Persinger (2003) found a massive collection of heterotypic cells, arranged as rosettes with normally shaped neurons, extending across the entire coronal area of the right hippocampus within the CA3-hilar region of one of the rats exposed to the low intensity complex sequence field.

Although we did not stain for dendritic processes, the pattern of arborizations (Greenough 1984, Barnes 1994) can be influenced by subtle shifts in the organizations of soma due to developmental anomalies. Structural anomalies within various regions of the hippocampus, induced by surgery or seizures, have been associated with alterations in spatial memory (Whishaw et al. 1994), conditioned taste aversion (Persinger et al. 1994) and contextual fear conditioning (Stewart & Persinger 2000). Other rats that had been exposed prenatally to the same complex sequence magnetic field that produced the increased quantitative anomalies in cell organization in the present study also showed alterations in spatial learning (McKay et al. 2002), open field behavior (Persinger & St-Pierre 2001), taste aversion, and contextual fear conditioning (St-Pierre & Persinger 2007).

We suspect a fundamental and shared mechanism may exist between: (i) The pattern of electrical stimulation that induced LTP in adult hippocampus slices (Rose et al. 1988), and, (ii) the increased incidence of cellular anomalies within the CA3/CA4-dentate regions of the hippocampal formation within the brains of rats that had been exposed prenatally to repeated presentations of this same 'LTP' pulse when it was applied as a magnetic field embedded within the complex sequence pattern. We suggest that the capacity for neurogenesis in the adult rat within this same region, particularly in response to either normal stimulation sufficient to produce long-term potentiation (Derrick et al. 2000) or electrical seizures (Parent et al. 1997), is not spurious (Fournier et al. 2007). Given the marked similarity in functional structure between the human and rat hippocampal formations (Gloor 1997), the possibility that such prenatal exposures might affect the human brain should be examined carefully.

Prenatal exposure to the complex sequenced magnetic fields was associated with significant intensity-dependent changes in blood chemistry. The strongest differences between rats that had been exposed to the different intensities occurred with the medium intensity group (median value 100 nT or 1 mG). These rats showed elevated activity for the enzyme aspartate aminotransaminase, lactic acid dehydrogenase activity, and alanine aminotransaminase but decreased concentrations of triglycerides.

Perinatal exposures to 0.5 Hz rotating magnetic fields with strengths 1000 times greater have also been associated with elevated levels of serum calcium but decreased activity of lactic dehydrogenase and alanine aminotransferase (Persinger et al. 1978). These rats had been exposed to field strengths between 1 microTesla and 1 milliTesla for two days before, the day of, and two days after birth. In the present study the rats exposed during their entire prenatal development to the 0.1 microTesla (1 mG) fields, in particular, showed increased levels of activity for these two enzymes.

In both studies blood urea nitrogen (BUN) in the rats exposed to either the stronger, 0.5 Hz rotating magnetic fields or the complex sequence fields (1000 times weaker) was lower than for the respective reference groups. Persinger and Mulligan (2001)

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have postulated that a rather simple process may occur. They assumed that: (i) The changes in the donation of the nitrogen from aspartate to citrulline were reflected by the deceased aminotransferase activity, and (ii) the alterations in the release of urea during conversion from arginine to ornithine were associated with lowered blood urea nitrogen levels. If these two processes occurred then the ornithine pathway would be implicated. There is empirical evidence for this supposition. Litowitz et al. (1997) have shown that ornithine decarboxylase activity does respond to the timing of complex magnetic fields with peak values around 10 microTesla.

The mechanisms of effect have been assumed to be related to some variant of currents due to the electric fields induced by the applied time-varying magnetic fields (Valentinuzzi 1961). All matter exhibits magnetism because all matter is composed of atoms and all atoms are composed of aggregates of protons around which electrons orbit and rotate. The living animal can be seen as a complex volume of time-varying magnetic fields being mediated by massive aggregates of ions, protons, and electron chains. Intuitively one might expect the processes that generate these fields would change quickly in the rat after conception. During the subsequent 21 days a single cell proliferates into a complex organism containing billions of cells. A single alteration in gene-expression or replication during this period within key progenitor cells could alter the cell line for the life of the organism.

As reported by Adey (1990), the typical cell membrane resistances are in the range of 3000–100,000 ohm/cm-squared while the extracelluar fluid's specific resistance is only about 50 ohm/cm-sq. Although extracelluar space occupies only 10% of the volume of brain space it carries about 90% of any imposed current. This extracelluar space is composed of ground substance a major component of which are the lecticans (Yamaguchi 2000), a family of chondroitin sulphates that includes aggrecan, versican, neurocan and brevican. They are closely associated with astrocytes (Brandtlow & Zimmerman 2000) that are connected by gap junctions. Different proteoglycans act as regulators of cell migration, axonal path finding, synaptogenesis, and structural plasticity. Expression of the brain-specific extracelluar matrix protein mediates the invasiveness of gliomas *in vivo* (Zang et al. 1998).

If lecticans are indeed the organizers of the extracelluar matrix, then even the smallest disruption in their organization could affect any of these processes. Usually one assumes that induced effects from applied magnetic fields occur homogeneously through tissue, including extracelluar space. As hypothesized by Persinger (1974) if some components (such as the polyanionic nets of the chondroitin sulphates) are *very* conductive, even weak current induction would affect those components because they would behave as 'electric sinks' if the applied magnetic fields are resonant with the natural frequency and mass of the molecule (Persinger 2006). Obviously the specific parameters for inductance and reluctance would be critical. Assuming 50 ohm/cm-sq and an electric field of about one microV (the value calculated by Faraday's law to occur within the area of the length of a 0.01 m exposed to a 100 nT (1 mG) field that is changing at 1 kHz (1 msec point durations with 100 microsec rates of shift between points), current values in the order of 1 nA/cm would occur; they are sufficient to affect cell growth according to Becker (1965).

The involvement of brevicans might explain the non-linear intensity effects observed in this study for the differences in blood chemistry and the histmorphological alterations, especially the odd patterns from myelin stains (Ogawa et al. 2001). These microstructures of ground substance might be imagined as a delicate matrix of conductors. Insufficient current does not affect them. Current parameters that match their capacitance, inductance, and reluctance allow optimal current induction that promotes potential conformational changes. Like any delicate circuitry, more intense current either destroys the 'wiring' or alters the molecular conformation from which different functions arise.

Acknowledgements

Thanks to Stan Koren and Chris Blomme for technical contributions. This research is dedicated to the memory of W. Ross Adey.

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